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Prevalence and Molecular Epidemiology of GB Virus C/Hepatitis G Virus Infection in Mongolia

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We studied the prevalence of GB virus C/hepatitis G virus (GBV-C/HGV) infection among 112 patients with liver disease and 121 blood donors in Ulaanbaatar, Mongolia. Reverse transcription and polymerase chain reaction were employed to detect GBV-C/HGV RNA using the specific primers derived from the 5′-untranslated region (5′-UTR) of the GBV-C/HGV genome. Nucleotide sequences of all positive samples for GBV-C/HGV RNA were determined. The sequences were analyzed by a molecular evolutionary method. Twenty-five (10.7%) of 233 people were positive for GBV-C/HGV RNA. Eight (6.6%), 11 (9.1%), and 30 (24.8%) blood donors were positive for GBV-C/HGV RNA, HBsAg, and anti-HCV, respectively, although 17 (15.2%), 65 (58.0%), and 64 (54.5%) patients with liver disease were positive for each viral marker. The prevalences of GBV-C/HGV RNA, HBV, and HCV in the patients were significantly higher than those in blood donors ($P < 0.05$). There was no significant difference in the prevalence of anti-HCV among people with and without GBV-C/HGV RNA, while the prevalence of HBsAg among people with GBV-C/HGV RNA was significantly higher than among those without GBV-C/HGV RNA ($P < 0.05$). The molecular evolutionary tree showed that GBV-C/HGV was a heterogeneous virus and all strains could be divided into 2 types. One is the same phylogenetic type as HGV, and the other is a new type that is different from GBV-C and HGV. ***J. Med. Virol. 52:143–148, 1997.***

KEY WORDS: GBV-C; HGV; GBV-C/HGV; Mongolia; 5′-UTR

INTRODUCTION

The cause of 10–20% of non-B, non-C hepatitis cases remains elusive following the discovery of hepatitis C virus (HCV) [Simons et al., 1995]. Recently, GBV-C and HGV were isolated independently by 2 different groups and were found to have a genome organized similarly to that of HCV [Leary et al., 1996; Linnen et al., 1996]. Further study showed that these 2 viruses are identical, so we refer to them as GBV-C/HGV in this study [Zuckerman, 1996].

A recent report showed that the risk of GBV-C/HGV infection appears to be higher in people with hepatitis B virus (HBV) or HCV than in people without HBV or HCV. In particular, approximately 20% of those with HCV infection were coinfected with GBV-C/HGV [Linnen et al., 1996]. Moreover, GBV-C/HGV RNA was detected among cases of fulminant hepatitis of unknown etiology in Japan [Yoshiba et al., 1995]. There remains a need to understand the natural history of GBV-C/HGV infection and its clinical significance, both in single infections and in relation to other hepatitis viruses.

Mongolia is known for its high prevalence of liver disease due to HBV and HCV [Tang, 1993]. The aim of the present study was to determine the prevalence of GBV-C/HGV among blood donors and patients with liver disease in Mongolia. A molecular epidemiological study of GBV-C/HGV in Mongolia was included and the nucleotide sequences of 5′-UTR were studied and analyzed using molecular evolutionary methods.

MATERIALS AND METHODS

Collection of the Serum Samples

Serum samples were collected from 112 patients and 121 blood donors in Ulaanbaatar, Mongolia, in August 1995 after informed consent. The patient group consisted of 13 patients with acute hepatitis (AH), 87 with chronic hepatitis (CH), 10 with liver cirrhosis (LC), and

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TABLE I. GBV-C/HGV, Anti-HCV, and HBsAg Among Mongolian Patients and Blood Donors

	No.	Age [yr] [Mean ± SE]	Gender [M:F]	ALT [IU/L] [Mean ± SE]	Positive for GBV-C/HGV RNA	HBsAg	Anti-HCV
Blood donors	121	30.2 ± 0.8	13:108	18.1 ± 2.2 [a]	8 (6.6) [a]	11 (9.1) [a]	30 (24.8) [a]
Patients with liver disease	112	27.7 ± 1.3	56:56	82.2 ± 14.3	17 (15.2)	65 (58.0)	61 (54.5)
AH	13	14.9 ± 3.5	8:5	359.3 ± 86.4	2 (15.4)	7 (53.8)	4 (30.8)
CH	87	27.9 ± 1.3	42:45	46.8 ± 6.9	13 (14.9)	49 (56.3)	48 (55.2)
LC	10	38.8 ± 4.7	5:5	42.6 ± 9.0	2 (20.0)	7 (70.0)	7 (70.0)
HCC	2	54.0 ± 5.0	1:1	171.5 ± 152.5	0 (0.0)	2 (100)	2 (100)
Total	233	29.0 ± 0.7	69:164	47.7 ± 7.0	25 (10.7)	76 (32.6)	91 (39.1)

[a] NS[b] [a]

Values are shown as mean ± SE.
Percentages are given in parentheses.
[a] $P < 0.05$.
[b] NS, not significant.

2 with hepatocellular carcinoma (HCC) (Table I). The clinical diagnosis was established by ultrasound, liver function tests, and measurements of alpha-fetoprotein levels.

Detection of HBsAg and Anti-HCV

HBsAg was detected by radioimmunoassay (Ausria II, Dinabot, Tokyo, Japan) and anti-HCV antibody was detected by second-generation enzyme immunoassay (EIA II, Ortho, Raritan, NJ). The limited amount of serum prevented other analyses, such as those with respect to HBeAg, anti-HBe, and HCV markers.

Extraction of RNA and Amplification by RT-PCR

RNA was extracted from 100 μl of serum with guanidinium thiocyanate, sodium acetate, and chloroform (SepaGene RV-R; Sanko, Tokyo). Extracted RNA was precipitated with isopropanol and washed with ethanol. The RNA pellet was dissolved in diethylpyrocarbonate-treated water containing 40 units of human placental ribonuclease inhibitor (Toyobo, Osaka, Japan).

cDNA was synthesized from the RNA by random priming using 200 units of Moloney murine leukemia virus reverse transcriptase (MMLV-RT; Gibco, Gaithersburg, MD) in a volume of 20 μl containing 1 × RT buffer (50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, and 10 mM DDT) and 600 μM of each dNTP (Promega, Madison, WI), and then incubated at 37°C for 60 minutes.

Amplification was carried out by nested PCR using oligomers corresponding to sequences in the 5′-UTR. For the 5′-UTR, the sense primer for the first round of PCR was 5gf2 (5′-GGTTGGTAGGTCGTAAATCCCG-GTCA-3′), and the anti-sense primer was 5gr4 (5′-GCGACGTGGACCGTACRTGGGCGT-3′). The sense primer for the second round of PCR was 5gf3 (5′-TGGTAGCCACTATAGGTGGGT-3′), and the anti-sense primer was 5gr4 (5′-GCGACGTGGACCGTA-CRTGGGCGT-3′). The sequence of a degenerate oligonucleotide is described using the standard IUPAC ambiguity code where R indicates A or G.

For the first PCR, 1 μl of cDNA was amplified with each oligonucleotide primer for 30 cycles of 94°C (denaturation) for 1 minute, 50°C (annealing) for 45 seconds, and 72°C (extension) for 1 minutes. For the second PCR, 1 μl of the first PCR product was amplified for 35 cycles of 94°C for 1 minute, 55°C were 45 seconds, and 72°C for 1 minute. These amplified products were analyzed by electrophoresis on 3% agarose gels, stained with ethidium bromide, and inspected under UV light.

Determination of Nucleotide Sequences

The seminested PCR products were sequenced bidirectionally using the dideoxy chain termination method (373A; Applied Biosystems, Foster City, CA). Twenty-five samples were used to determine the 5′-UTR nucleotide sequences.

Molecular Evolutionary Analysis

The number of nucleotide substitutions per site was estimated by the 6-parameter method and phylogenetic trees were constructed by the Neighbor-joining method using the numbers of the substitutions [Gojobori et al., 1982; Saito et al., 1987]. To confirm the reliability of the phylogenetic tree, bootstrap resampling tests were carried out 100 times [Felsenstein, 1985]. These analyses were conducted using the computer program ODEN, version 1.1.1 [Ina, 1994].

Statistical Analysis

Student's t-test and Fisher's exact test were used for statistical analyses.

RESULTS

Among the 233 individuals studied, 25 (10.7%), 76 (32.6%), and 91 (39.1%) were positive for GBV-C/HGV

TABLE II. Comparison of Characteristics According to GBV-C/HGV RNA in Mongolian Patients and Blood Donors

	GBV-C/HGV RNA		
	Positive	Negative	*P*-value
No.	25	208	
Gender [M:F]	11:14	58:150	NS
Blood donors	0:8	13:100	NS
Patients with liver disease	11:6	45:50	NS
Age (yr; mean ± SE)	27.2 ± 3.3	29.2 ± 0.7	NS
ALT (IU/L; mean ± SE)	53.2 ± 14.3	47.1 ± 7.6	NS
Positive for			
HBsAg	14 (56.0)	62 (29.8)	$P < 0.05$
Anti-HCV	12 (48.0)	79 (38.0)	NS[a]

Values are shown as mean ± SE.
Percentages are given in parentheses.
[a]NS, not significant.

RNA, HBsAg, and anti-HCV, respectively (Table I). The prevalence of GBV-C/HGV RNA was significantly lower than the prevalence of anti-HCV or HBsAg ($P < 0.05$), while there was no significant difference in the prevalence of HBsAg and anti-HCV. Among the 121 blood donors, 8 (6.6%), 11 (9.1%), and 30 (24.8%) were positive for GBV-C/HGV RNA, HBsAg, and anti-HCV, respectively. Among the 112 patients with liver disease, 17 (15.2%), 65 (58.0%), and 61 (54.5%) were found to have GBV-C/HGV RNA, HBsAg, and anti-HCV in their sera, respectively. GBV-C/HGV RNA was detected in the sera of 2/13 (15.4%), 13/87 (14.9%), 2/10 (20.0%), and 0/2 (0%) of the patients with AH, CH, LC, and HCC, respectively. The prevalence of GBV-C/HGV, HBV, and HCV in patients was significantly higher than in blood donors ($P < 0.05$).

The serum alanine aminotransferase (ALT) level of blood donors was significantly lower than that of patients with liver disease, although there was no significant variation in ALT level according to diagnosis of liver disease.

There was no significant difference in age, gender, serum ALT level, or prevalence of anti-HCV between the groups with and without GBV-C/HGV RNA. But prevalence of HBsAg among the group with GBV-C/HGV RNA was significantly higher than in those without GBV-C/HGV RNA (Table II).

Among the 233 individuals, 3 (1.3%) were found with single GBV-C/HGV RNA. All had normal ALT level. Single infection with HBV or HCV occurred in 45/233 (19.3%) and 62/233 (26.6%), respectively. The serum ALT level and its rate of elevation (above 30IU/l) did not differ significantly between patients with single HBV infection and patients double infected with HBV and GBV-C/HGV. Similarly, the serum ALT level did not differ between patients with single HCV infection and those infected with both HCV and GBV-C/HGV (Table III).

To confirm the detection of GBV-C/HGV RNA, we determined the nucleotide sequences and aligned these sequences within the 5′-UTR of the GBV-C/HGV genome (Fig. 1). These alignments showed that our sequences from Mongolia differ from those of GBV-C isolated from West Africa, with a homology of 80.3–83.9%. Furthermore, some of the strains were similar to HGV from the United States, with 86.1–97.1% homology.

We also undertook molecular evolutionary analyses to investigate the molecular epidemiology of GBV-C/HGV in relation to the known sequences of GBV-C and HGV. According to the phylogenetic tree of the 5′-UTR, it appeared that every strain could be divided into 2 types. Sequences from 6 isolates, M263-CH, M287-CH, M79-CH, M25-LC, M102-CH, and M115-BD, were within the same phylogenetic cluster as HGV. The other 19 isolates, M186-CH, M320-AH, M358-CH, M60-CH, M351-CH, M345-CH, M331-CH, M41-LC, M82-CH, M329-CH, M102-BD, M117-BD, M75-BD, M92-BD, M91-BD, M104-BD, M98-CH, M323-AH, and M88-BD represented new clusters differing from GBV-C and HGV (Fig. 2).

To estimate further reliability of the phylogenetic tree, bootstrap analysis was also carried out. The sequences were resampled 100 times and the phylogenetic trees were reconstructed each time. The results of the bootstrap analysis of both regions supported the conclusion that the strains isolated from the sera of the Mongolian patients could be divided into 2 types (Fig. 2).

TABLE III. Viral Status and ALT Among Mongolian Patients and Blood Donors

Viral status for						
GBV-C/HGV	HBsAg	Anti-HCV	No.	ALT[IU/L] [Mean ± SE]	ALT [>30 IU/L]	
+	−	−	3 (1.3)	4.7 ± 2.3	0/3 (0.0)	
−	+	−	45 (19.3)	82.6 ± 22.7	13/34 (38.2)	NS*
−	−	+	62 (26.6)	35.1 ± 6.9	11/36 (30.6)	
+	+	−	10 (4.3)	136.2 ± 61.1	4/6 (66.7)	NS
+	−	+	8 (3.4)	38.9 ± 11.1	3/6 (50.0)	
−	+	+	17 (7.3)	78.8 ± 22.2	9/14 (64.3)	
+	+	+	4 (1.7)	49.0 ± 30.0	1/2 (50.0)	
−	−	−	84 (36.1)	26.1 ± 11.1	4/84 (5.8)	
Total			233	49.8 ± 7.3	44/103 (42.7)	

Values are shown as mean ± SE.
Percentages are given in parentheses.
*NS, not significant.

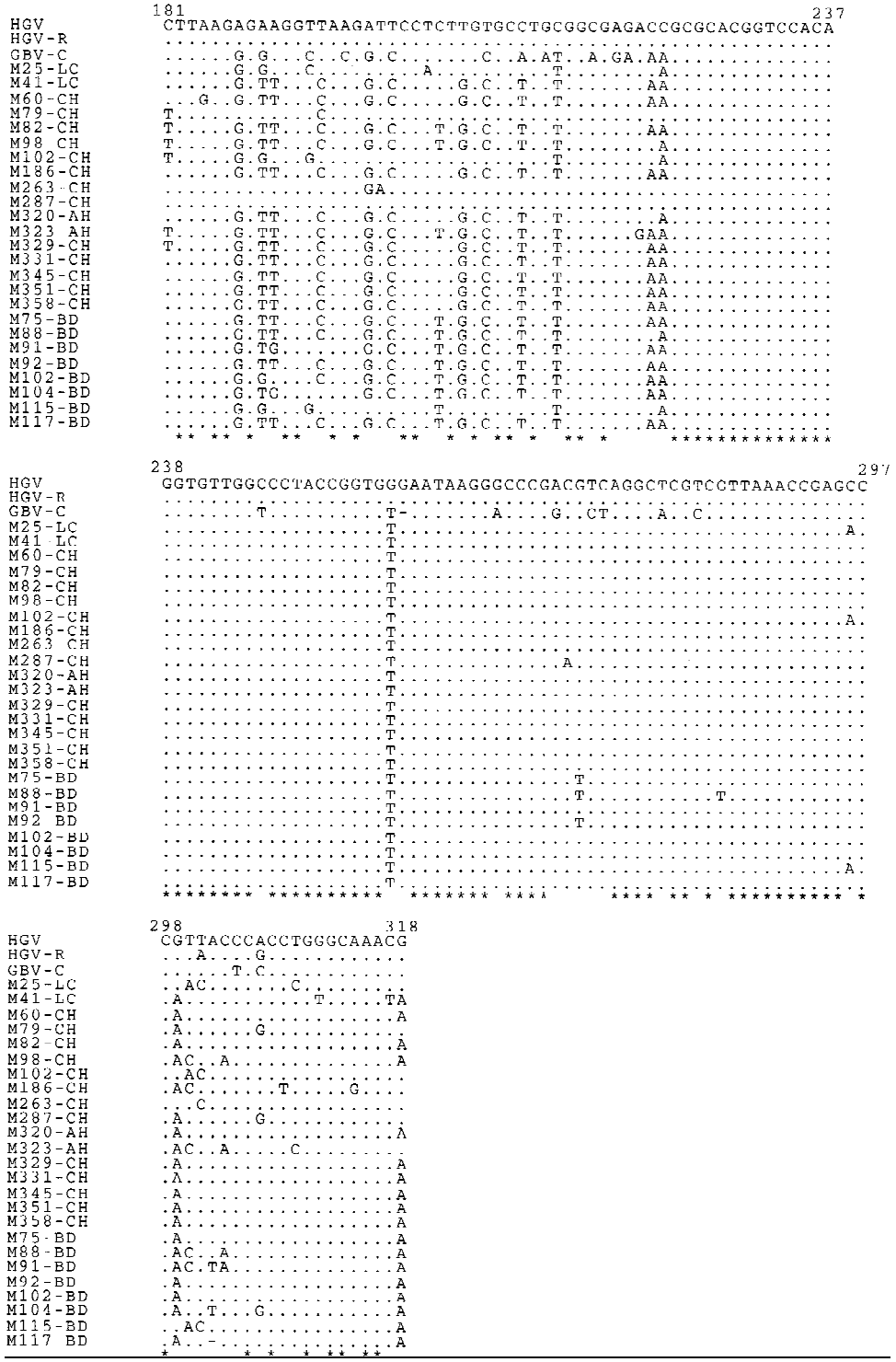

Fig. 1. Alignment of GBV-C/HGV 5′-untranslated subgenomic region sequences. The clones obtained in this study are represented as M: Mongolia, AH: acute hepatitis, CH: chronic hepatitis, LC: liver cirrhosis, and BD: blood donor, followed by the identification number. GBV-C, HGV, and HGV-R sequences were obtained from DDBJ/Genbank/EMBL (accession numbers U36380, U44402, and U45966, respectively). The number on the alignment was named according to HGV.

DISCUSSION

The prevalence of GBV-C/HGV was investigated in Ulaanbaatar, Mongolia, an area that is known for its high prevalence of liver disease [Tang, 1993]. We estimated the prevalence of GBV-C/HGV by RT-PCR with specific primers derived from the 5′-UTR and carried out molecular evolutionary analyses.

GBV-C/HGV RNA was significantly less common than anti-HCV or HBsAg in the sera of the Mongolian

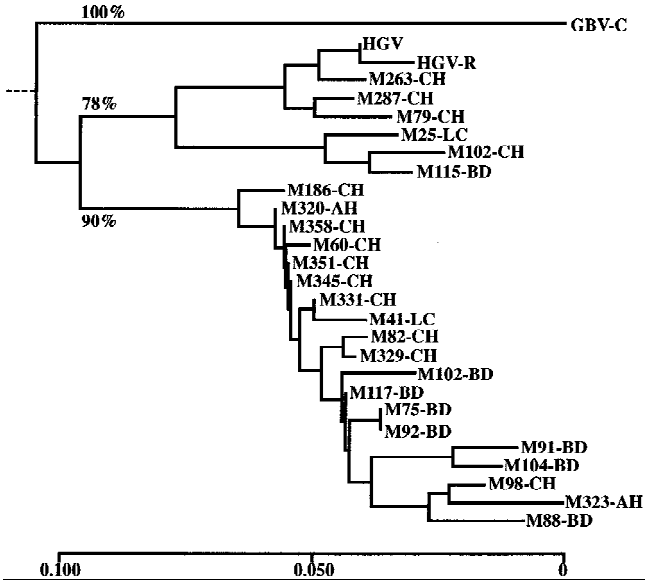

Fig. 2. Phylogenetic tree of the GBV-C/HGV 5′-untranslated subgenomic region. These isolates are clustered into two phylogenetic branches, one with HGV and HGV-R, and another with putative new clusters at a separate branch. The numbers beside the true roots are the results of bootstrap analysis. The horizontal bar at the bottom indicates the number of nucleotide substitutions per site.

patients with liver disease. This observation raised two possibilities. First, HCV and HBV were introduced into Mongolia before GBV-C/HGV. Second, some patients with GBV-C/HGV recovered spontaneously. With regard to the first possibility, phylogenetic trees showed a large genetic distance among virus strains, indicating that GBV-C/HGV may have been introduced into Mongolia long ago. Regarding the second possibility, Linnen et al. [1996] reported that GBV-C/HGV RNA was not detected after the onset of infection in a naturally resolved case. Hence the differences between the prevalences of HBsAg, anti-HCV, and GBV-C/HGV RNA may be due to spontaneous recovery from GBV-C/HGV infection. It is difficult to confirm these differences because reliable serological assays for GBV-C/HGV are not available. Development of an antibody assay for GBV-C/HGV is needed.

The prevalence of GBV-C/HGV RNA in Mongolian patients with liver disease (17/112, 15.2%) and blood donors (8/121, 6.6%) is higher than that in Japanese patients (10/203, 4.9%), European patients (35/290, 12.1%), and Japanese blood donors (1/200, 0.5%) [Linnen et al., 1996; Orito et al., 1996]. There was no significant difference in clinical characteristics, including anti-HCV positivity and serum ALT level between the patients with and without GBV-C/HGV RNA. The prevalence of HBsAg among people with GBV-C/HGV RNA was significantly higher than in those without GBV-C/HGV RNA ($P < 0.05$), although Linnen et al. (1996) reported previously that the prevalence of GBV-C/HGV RNA with HBsAg was lower (7/72, 9.7%) than that with anti-HCV (18/96, 18.8%). Interestingly, the prevalence of HGV, HBV, and HCV in patients was significantly higher than in blood donors ($P < 0.05$). In Mongolia, there are many patients with chronic liver disease who acquired hepatitis by a transfusion-associated route or from unsterilized needles and syringes due to economical conditions. Hence GBV-C/HGV infection was probably spread in a way similar to HBV [Wu et al., 1995].

Note that there were 3 cases with a single infection with GBV-C/HGV. Serum ALT levels in the patients were normal. Moreover, ALT level in those infected only with HBV did not differ significantly from that of the patients infected with both HBV and GBV-C/HGV. The serum ALT level of the patients in whose sera only anti-HCV was detected also did not differ significantly from that of the patients in whose sera both anti-HCV and GBV-C/HGV RNA were detected. These findings raised three possibilities. First, GBV-C/HGV is not a main cause of non-B, non-C hepatitis in Mongolia. Second, this virus might cause even less liver dysfunction than HBV or HCV, and third, it has no influence on the progression of liver disease in patients with HBV or HCV. Previous reports suggested that GBV-C/HGV is an agent of acute hepatitis and is not a cause of severe hepatitis, although GBV-C/HGV RNA was detected in the patients with fulminant hepatitis of unknown etiology in Japan [Yoshiba et al., 1995; Kao et al., 1996; Kuroki et al., 1996]. In our study there were no cases of fulminant hepatitis.

A phylogenetic tree of the 5′-UTR showed that GBV-C/HGV in Mongolia is a heterogeneous virus and that every strain we isolated can be divided into 2 types. One type is the same phylogenetic type as HGV, originally described from the United States, and the other is a putative new type which is quite different from the GBV-C originally isolated from West Africa. This was also confirmed by using bootstrap analysis. Thus GBV-C/HGV should be classified into three types, two of which were found in Mongolia.

The natural history and clinical significance of GBV-C/HGV related hepatitis require further study.

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